Aus dem Pathologischen Institut der Universität Basel
(Vorsteher: Prof. Dr. A. Werthemann)

aus der Chirurgischen Universitätsklinik Basel
(Vorsteher: Prof. Dr. R. Nissen)

Arbeit unter Leitung von PD Dr. F. Gloor und PD Dr. E. C. Yasargil

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MORPHOLOGICAL STUDIES OF THE KIDNEY FOLLOWING POLYBRENE APPLICATION

INAUGURAL-DISSERTATION

zur Erlangung der Doktorwürde der medizinischen Fakultät
der Universität Basel

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INTRODUCTION

The purpose of this paper is to illustrate the possibility of producing nephrotoxic effects in animals using Polybrene. Polybrene (hexadimethrine bromide) is a polymeric quarternary ammonium salt, a poly-1,5-dimethyl-1,5-dizaundecamethylene dimethobromide.

Only but a short time ago this substance was quite widely used as a substitute for protamine sulphate for heparin neutralization as used in open heart surgery. Cardiac surgery involving extracorporeal circulation requires 20—25 cmm heparin per 500 cmm blood. While one is dealing with 4—5 litres of blood and that heparin loses its effect considerably after 2 hours, a significant amount of heparin is often needed, particularly in order to prevent microcirculatory coagulation. Immediately upon separation from the heart-lung apparatus, heparin needs to be neutralized. This has been classically accomplished by using 1.3—2.5 mg protamine sulphate per mg heparin. Unpleasant side effects such as dangerous hypotension and the possibility of protamine sulphate overdosage made one search for a more stabile substance.

Studies of Polybrene were first published by Preston in 1953. This was followed by a more detailed report using dogs by Preston and Parker. Subsequently, numerous experimental and clinical investigatons followed (Preston et al., Weiss et al., Andersen et al., Kimura et al.). Lillehei et al. was then the first to use it clinically. Thus far only three clinical reports on renal toxicity have appeared (Pate et al., Haller et al., and Yasargil et al.). At the Surgical Clinic at the University of Basle this substance was implicated as a contributing factor in a number of post-operative deaths presenting a clinical picture of renal failure, all having been given up to 5 mg/kg Polybrene during open heart surgery

(Yasargil). The histopathological examinations of the kidneys at autopsy, however, revealed only minimal findings not compatible with the clinical situation. With our experimental study we hope to define the exact nature of this renal toxicity.

EXPERIMENTAL PROCEDURE

For histological and histochemical analysis 6 groups of 3 adult female rats each were subjected to the following dosage schedule: 1) Control, 2) 2 mg/kg, 3) 4 mg/kg, 4) 8 mg/kg, 5) 16mg/kg, 6) 16 mg/kg. A second series of adult female rats were used in the following manner, 3 rats in each group: 1) Control, 2) 8 mg/kg, 3) 16 mg/kg, 4) 32/kg.

All injections were made intravenously using the veins of the tail. The animals were kept on a normal laboratory diet with water ad libitum. After 3 days, the animals of the two series were in good health and showed no signs of renal insufficiency. Liver and kidney tissue were prepared for histochemical enzyme analysis using a Dittes-Duspiva cryostat. Each kidney and liver was frozen within 5 minutes after killing the animal. Care was taken to sacrifice the animals simultaneously and immediately mount the organs for frozen section. The organs, including the controls, were mounted along side each other on the same cryostat table, enabling convenient comparison. The following enzymes were examined: acid and alkaline phosphatase (method of Goessner), adenosin triphosphatase at pH 7.2 (method of Padykula and Herman) and at pH 9.4 (method of Wachstein and Meisel), succinate dehydrogenase, lactate dehydrogenase, and cholin dehydrogenase (method of Glogner and Goessner). Tissue samples of each organ were fixed in Susa (formol-sublimat-trichloroacetic acid-glacial acetic acid), embedded in paraffin and finally stained with haemalaun-eosin, Astrablue-PAS for glycogen, acid and neutral mucopolysaccharides, Kossas silver stain for calcium phosphate and Masson-trichrom. In some cases material was fixed in neutral formol and stained for fat.

RESULTS

Renal Morphology of Adult Female Rats: Series 1 and 2.

Macroscopic Findings: The kidneys were of normal weight, the surface smooth, the capsule stripping easily. On cross section the cortex appeared uniformly red-brown while the medulla appeared somewhat paler. The cortico-medullary junction and the outer medullary zone were normal in animals receiving 8 mg/kg and 32 mg/kg, the outer medullary zone was pale and prominent throughout both kidneys of each animal.

Microscopic Findings: 1) Animals receiving 8 mg/kg: the glomerular loops appeared unchanged. The lumina of the cortical tubules were narrow and the brush-border of the epithelium intact. The medullary tubules appeared unchanged except in one case in which it was noted an increase in mitotic activity of the tubular epithelium. Occasionally, a single tubular epithelial cell showed necrosis. These latter changes were confined to the thick ascending portion of Henle's loop in the transitional zone between cortex and medulla. Occasional epithelial cells had desquamated into the lumina while granulated hyaline casts were noted in Henle's loop. The interstitial tissue and blood vessels were noncontributory. 2) Animals receiving 16 mg/kg: Here again the glomerular tufts showed no evidence of increased cellularity nor was the basement membrane thickened. The proximal cortical tubules appeared unchanged and the brush-border was intact as shown with PAS. The distal convoluted tubules were somewhat widened, their epithelium intact. The interstitial tissue of the cortex and the blood vessels of the entire kidney were noncontributory. A significant finding throughout the parenchyma was a distinct dilatation of the descending straight portion of the proximal tubules. Here the PAS-positive brush-border was of reduced intensity. In all cases, a severe pathological change manifested itself and primarily involving those tubules of the outer medullary zone. The epithelium of the ascending thick portion of Henle's loop showed increased basophilic staining and numerous pyknotic nuclei. In many areas, epithelial necrosis was abundant while in other areas an increased

mitotic activity of the tubular epithelium was evident. With the Astrablue-PAS the necrotic epithelium stained blue. Considerable deposition of the calcium phosphate within the necrotic tubules of this region also occured as shown with the Kossas silver stain. Aside from the desquamated epithelium found within the lumina of Henle's loop, the presence of granulated hyalin casts were noted. The latter stained PAS-positive. The interstitium of the damaged outer medullary zone showed slight mononuclear cell infiltrates and a few polynuclear elements. The inner zone of the medulla with its thin portion of Henle's loop and collecting tubules showed no epithelial necrosis and remained unchanged. 3) Animals receiving 32 mg/kg: These kidneys showed an increased involvement of the cortex. Occasionally, a few leucocytes were seen within the capillaries of the glomerulum. In several places a discrete thickening of the glomerular basement membrane could be noted. The proximal tubules were dilated, its epithelium flat while the brush-border still remained intact, but stained less intensively with PAS as the controls. Within the lumina of these dilated proximal tubules numerous homogenous PAS positive cylinders were noted. The distal tubules of the renal cortex were likewise dilated. The interstitium of the cortex was unaltered. Within the tubular epithelium there was no evidence of protein accumulation nor deposition of fat. The more pronounced changes were to be seen in the outer medullary zone. The tubuli were considerably dilated, their epithelium showing extensive necrosis which had gathered within the lumina (Fig. 1). Several tubules appeared only as thin tubes with nothing remaining of the epithelium but the basement membrane. PAS-positive granulated cylinders were found in the dilated tubules of the outer medullary zone as well as within the thin descending portion of Henle's loop and several collecting tubules in the inner medullary zone. The interstitium at the cortico-medullary junction showed minimal lymphocytic and plasma cell infiltration. However, toward the inner medullary zone and papillae the interstitium appeared unchanged.

Histologically, the livers were noncontributory.

Histochemical Findings: In general, it could be shown that sligth changes occured at the outer medullary zone after 8 m/kg

and marked changes after 16 mg/kg and 32 mg/kg application. The reaction detecting the presence of cholin dehydrogenase illustrated a uniform relationship to the dosages given. At 8 mg/kg an inhibition of this enzyme was detectable, beginning in the ascending thick portion of Henle's loop where the enzyme appeared to be totally absent. This disturbance extended further proximally to include the thin portion of Henle's loop (Fig. 2). The reaction for succinate dehydrogenase was considerably inhibited in the entire loop of Henle. To a lesser degree this was also true of lactate dehydrogenase. The lumina of the thin portions of Henle's loop were frequently entirely obstructed by necrotic debris associated with extensive dilatation of these tubules, a dilataion which extended proximally into the straight portion of the proximal tubules situated in the outer medullary zone. Occasionally, the epithelium of the proximal tubules appeared damaged as shown by the destruction of the brush-border evidenced by the disappearance of alkaline phosphatase activity. On the basis of this enzyme activity, it could be shown that the necrotic debris found within the thick portion of Henle's loop reacted intensively to alkaline phosphatase activity. The high dosages of 32 mg/kg produced a disappearance of acid phosphatase in the entire loop of Henle. The cellular debris situated within the loop showed acid phosphatase activity (Fig. 3). All of these changes were evenly distributed throughout the renal parenchyma.

Only by the administration of 32 mg/kg could an obvious damage to the liver be shown as illustrated by the inhibition of adenosin triphosphatase. An additional disturbance was indicated by the appearance of alkaline phosphatase activity in the bile canaliculi. This illustrates an inhibition of liver clearance for alkaline phosphatase.

DISCUSSION

Our experiments have shown that Polybrene, administered in large dosages, damages the rat kidney. The changes demonstrated were characteristically located. The pathological alterations and

the loss of enzymatic activity were most pronounced at the outer medullary zone and were evenly distributed throughout both kidneys of each animal. These changes primarily involved the ascending thick portion of Henle's loop and to a lesser extent the descending straight portion of the proximal tubules of the renal cortex.

Thus far only clinical suggestion has been given to the possibility of renal damage based upon rather insufficient morphological support (Pate et al., Haller et al. and Yasargil et al.).

Even with a smaller dosage of 8 mg/kg discrete changes were documented. Damage to the tubular epithelium could be construed from the presence of increased mitotic activity, an occasional necrosis and the minimal reduction of enzymatic activity.

The general histological appearance of the kidney following the application of high dosages (16 mg/kg and 32 mg/kg) of Polybrene resulted in severe damage to the tubular epithelium. The pathology appeared limited to the distal tubules and in that area considered as the transitional zone between cortex and medulla and the outer medullary zone. Numerous tubular lumina were filled with massive necrotic epithelial debris. Consequential to this, it could be shown that the proximal tubules were somewhat dilated, not themselves being primarily disturbed as evidenced by an intact brush-border and an intact enzymatic activity. However, the latter did show with increased dosage a decrease in alkaline phosphatase activity, hinting a minimal embarrassement to these tubules took place as the renal damages progressed. As the proximal dilatation became pronounced in the later stages, proximal tubular necrosis was evident with total disappearance of the brush-border and total absence of alkaline phosphatase activity.

The use of enzyme histochemical analysis helped us to illustrate more clearly the exact location of any pathological change that might have been responsible for renal dysfunction. The almost total inhibition of the two enzymes, succinate dehydrogenase and lactate dehydrogenase, in the descending straight portion of the proximal tubules and the ascending thick portion of Henle's loop may be considered as suggestive evidence of irreversible damage to these tubular epithelial cells. The early disappearance of succinate dehydrogenase must be considered

as a manifestation of irreversible damage to the mitochondria, since succinate dehydrogenase is considered to be intimately bound to the mitochondrial structures. The enzyme lactate dehydrogenase, found in the cytoplasm, will show depletion upon disturbance or destruction of the cytoplasmic membrane. This explains why lactate dehydrogenase inhibition occurs later than the inhibition of succinate dehydrogenase. As the mitochondria are destroyed a blockage of energy production occurs which precedes cell death and cell membrane destruction.

Larger dosages of Polybrene also showed decreased activity of acid phosphatase and adenosin triphosphatase of the tubular epithelium in the outer medullary zone. The descending straight portion of the proximal tubules, also found within this zone, showed diminished presence of alkaline phosphatase activity so characteristic of the brush-border. This same alkaline phosphatase activity could be seen as part of the necrotic debris found within the thin portion of Henle's loop.

Most known nephrotoxic agents first appear to damage the proximal convoluted tubules. The toxic substance is initially filtered by the glomerular tufts. The ultra-filtrate becomes concentrated by the proximal tubular epithelium, thus often resulting in epithelial necrosis as shown, for example, by applying $HgCl_2$ (sublimat). Other substances are not reabsorbed by the proximal tubules and pass into Henle's loop. Here within this loop, these substances become concentrated by the hair-pin-counter-current system and may cause considerable damage to the epithelial cells. The ascending thick portion of Henle's loop with its high-geared oxidative metabolism is far more susceptible to the influence of tubular concentrates than the thin portion of Henle's loop. It has been established that the rat, especially the female rat, concentrates her urine up to four times more than man. With this fact, we can help to explain why Polybrene has a toxic effect in rats, but fails to show visible alteration in man.

These kidney changes are similar to those that were caused by using halogenated quinoline and quinaldine derivatives (Meier-Ruge) except that the changes with Polybrene were more diffuse and uniform. The changes, observed in our experiments, occuring in the outer medullarly zone could possibly be explained by an

imbalance of the hair-pin counter-current concept (Wirz and Kuhn) enabling the concentration of Polybrene to reach toxic levels within the tubules. It would follow that the greater the tendency toward urinary concentration, the greater the tubular damage. An important additional factor as exsiccosis would surely make the situation all the more critical. In this way we propose that the post-operative state with an imbalance in electrolytes and tissue water induced by operative stress, blood transfusions, etc. acts as a potentiating factor for the provocation of Polybrene damage in man's kidney. However, our animal experiments show, that Polybrene alone without the additional factors produces toxic effects in the kidney. We realize, however, we must be extremely cautious interpolating from our animal experiments that Polybrene has toxic effects on human kidney.

It is not probable that the toxic effect as measured by the enzymes studied is of a primary nature. Thus, all reactions showing enzyme inhibition would be considered as secondary manifestations. The enzyme depletions are probably consequential to disturbed metabolic processes, involving enzymes other than those studied.

SUMMARY

An attempt was made to substantiate the fact morphologically that the substance Polybrene (Hexadimethrine bromide), a heparin neutralizer, possesses nephrotoxic effects and to show exactly the nature of this action. It could be shown histologically and with histochemical analysis that Polybrene causes metabolic alterations in the tubules of the outer medullary zone. Tubular epithelial necrosis and enzyme depletion were most pronounced in the ascending thick portion of Henle's loop. This disturbance is probably nothing more than a secondary manifestation of some yet unknown intra-cellular metabolic derangement.

Generic and Trade Name of Drug: Hexadimethrine bromide — Polybrene

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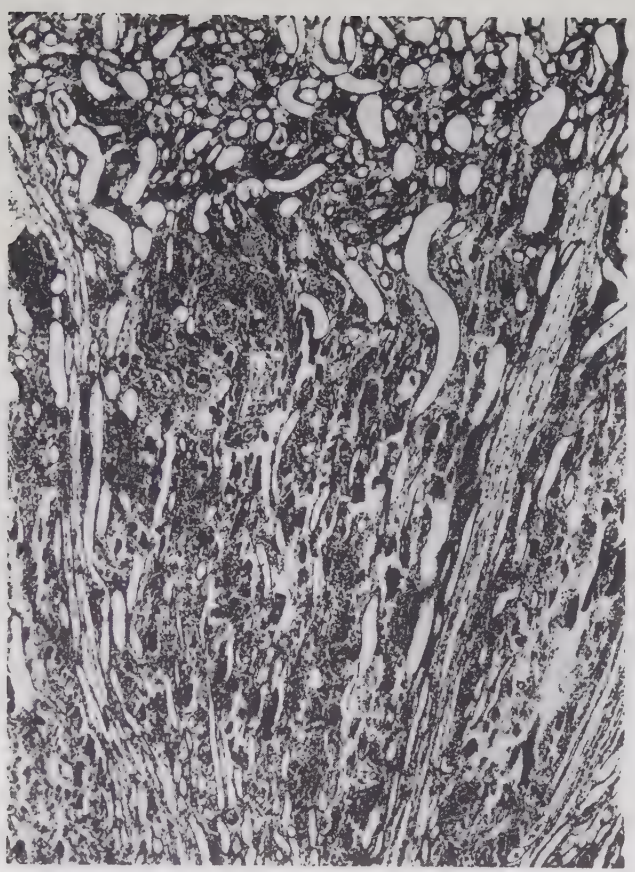

FIGURE 1

Rat kidney: Epithelial necrosis of Henle's loop, interstitial inflammation and dilatation of the straight descending portion of the proximal tubules. Haemalaun-Eosin, 60 X.

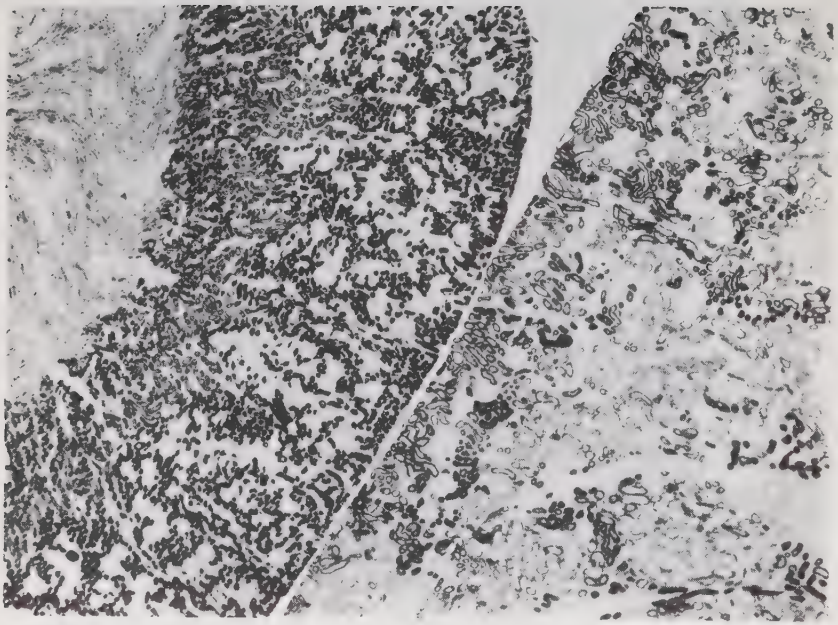

FIGURE 2

Rat kidney: Cholin dehydrogenase activity after 120 minutes incubation. 3 days after 32 mg/kg Polybrene i. v. Significant reduction of enzymatic activity in the cortex (right in Figure) in contrast to the control kidney (left in Figure). 20 X.

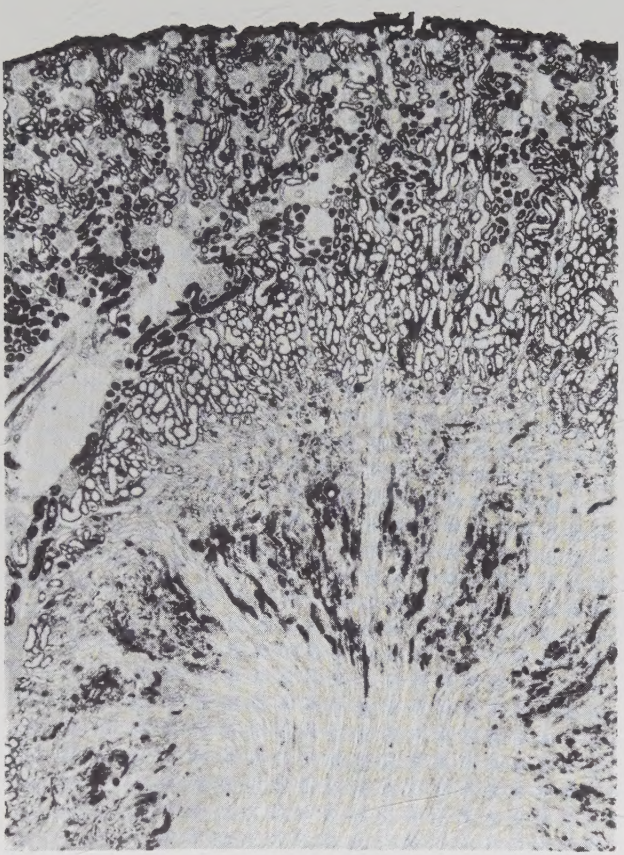

FIGURE 3

Rat kidney: Acid phosphatase. 3 days after 32 mg/kg Polybrene i. v. Significant loss of enzymatic activity in the outer medullary zone. Cylinders with acid phosphatase activity within the loop of Henle. Spotty enzymatic depletion of the cortical tubules. 23 X.